The Rufford Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Clara Andrea Ortiz Alvarez
Project title	Marine otter (*Lontra felina*) monitoring along the Peruvian coast and population genetics using mitochondrial DNA markers
RSG reference	20079-1
Reporting period	June 2016 – June 2017
Amount of grant	£ 5000
Your email address	Claraoa.2502@gmail.com
Date of this report	15/06/17

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Undertaking workshops to coastal communities about marine environment and conservation				We performed 15 workshops. Nine of these were given to primary schools with a total attendance of 325 children between ages 6 and 9 years. One talk was given at a kindergarten with an attendance of 15 children. Finally, five talks were given to industrial fishers, with a total attendance of 77 participants. In all workshops, the main topics were marine biodiversity, threats conservation status of marine fauna and the importance of marine protected areas.
Sampling along the Peruvian coast: three zones North, Centre and South				We surveyed 25 locations along the northern, central and southern Peruvian coast (north= six, centre= eight, south =11).
Performing DNA analysis of scats samples in Peru				We sequenced 96 marine otter scats samples employing two mitochondrial markers (control region and cytochrome b). We obtained sequences from all locations samples from the three target zones.
Training of Peruvian professionals in molecular techniques, analysis and interpretations of results				As part of this project I was accepted and participated in the XI International Workshops of Conservation genetics: genes, ecology and society. The workshop is held yearly by the Conservation Genetics Network (REGENEC), a Latin-American society whose aim is to reinforce the application of population genetics into conservation issues in Latin America.
Partner with the National University of Santa and Private laboratories				A partnership has been established through a collaboration agreement signed May 2017 between Pro Delphinus and the University of Santa. Furthermore, we also collaborated

				with the private laboratory BioAL Sac to perform the laboratory analysis. In both case the possibility to develop future project is open.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

Marine otters' habitat physical characteristics: One of the main project difficulties and challenges was the access to some sampling areas. Along the Peruvian coast, there are many rocky areas that could be considered ideal for marine otters. However, these areas are usually at the end of high and steep cliffs, which are difficult to access without specialised equipment. Nevertheless, we were able to sample some of these areas in Ancash (northern coast) and Arequipa (southern coast) given the abilities of one team member with wide knowledge these areas and personal abilities. As an alternative, when terrain was too steep, we performed surveys through observations from top of the cliff. Even though this limited the possibility of collecting samples it did allow for the detection of marine otters. Another challenge we faced was the limited public transportation available to reach the sampling areas, especially in the region of Ica (central coast). However, we were able to contact private drivers who helped us during the surveys.

Bureaucratic permits and processes: One of the biggest issues was the slow bureaucratic process we had to follow to establish the collaboration with the University of Santa to perform the laboratory analysis. The collaboration process between the NGO Pro Delphinus and the University of Santa began mid 2016 but was not signed until May 2017. To overcome this hurdle we contacted the private laboratory BioAl and its director Dr Monica Santa Maria. Even though our marine otter project was not the focus of the laboratory, Dr Santa Maria agreed to work with us and process all the samples and demonstrated a strong interest and commitment to the project. Despite not being able to work in collaboration with University of Santa during this project, now through the established agreement with the Pro Delphinus we will have the opportunity to continue this project in the future.

Laboratory analysis: The standardisation of the DNA extraction protocols was one of the main challenges and difficulties. As scats are considered to have low concentrations of high quality DNA, many trials employing commercial DNA extraction kits and "homemade" methods were performed before getting results sufficient to continue with analysis. The next step, the polymerase chain reactions (PCR), did also require time to establish a standardized protocol, especially as we were using two markers (cyt-b and controls region). However, we were able to obtain an acceptable concentration of DNA from 96 samples per mitochondrial marker. All samples were delivered to Macrogen USA for sequencing. Despite the longer than expected time for laboratory processing, we were able to obtain DNA from scat samples without the need to send them to laboratories out of the country. Furthermore, with this experience we now have a baseline for performing additional nuclear DNA analyses that will be developed in collaboration with the University of Santa.

Environmental talks: Due to logistical constraints, we were not able to conduct talks in all zones visited during the project. We were, however, able to reach more than 300 children in the talks we conducted, particularly in the southern zone. We have kept in contact with three schools to develop future projects. Regarding fishers, we gave talks to 77 industrial fishers from different ports along the country.

3. Briefly describe the three most important outcomes of your project.

1. Monitoring of marine otter along its Peruvian distribution:
   During the project period, we surveyed the Peruvian population of marine otters along its distribution from Ancash to Tacna. Eleven locations were surveyed in southern Peru from the region of Tacna to Arequipa, eight locations in Central Peru including the regions Ica and Lima; and in northern Peru, six locations in the region of Ancash. Within these locations we completed 20 transects surveying 141.34 km of coastline. Two locations were surveyed by boat (Ancon and Pucusana, in the region of Lima) and two others, Cerro Azul and Punta Corrientes, were surveyed specifically for collecting scat samples. The presence of marine otters was registered in 88% of transects; scats represented 76% of the signs registered as marine otter presence, 14% were sightings and 10% was food waste (i.e. fish remains).

   Human disturbance was registered in all locations visited, as either structure, fishers or nearby roads. In 15% of transects, all sections had human disturbance, in two of them no marine otter presence was detected. The increasing expansion of coastal urban areas was noteworthy, especially in Lima. Construction is changing the landscape and polluting the environment due in part to inadequate drainage. Furthermore, the number of domestic animals such as cats and dogs may represent a threat to marine otter health. We found the large amounts of human produced garbage and debris along the coast, including in protected areas, also particularly noteworthy. This included mainly the presence of solid wastes like Styrofoam, plastic and glass.

2. DNA sequencing of marine otters from northern, central and southern locations in Peru:

   We collected 138 marine otter scat samples during field excursions, genomic DNA was extracted from 96 samples using the QIAMP DNA stool minikit. Using polymerase chain reaction (PCR), two mitochondrial DNA markers were amplified the control region and cytochrome B, of approximately 300 and 700 basepairs, respectively. Despite this, it was not possible to obtain high quality DNA from all samples. All 96 samples were delivered to Macrogen USA to sequence the forward and reverse strands for each marker. Macrogen delivered 192 sequences per marker, from which 46% are appropriate for further analysis.

   In partnership with the NGO Pro Delphinus we were able to complete a formal collaborative agreement with the National University of Santa, located in the region of Ancash. This collaboration will facilitate the development of

future projects on conservation genetics, as it possesses one of the well-equipped laboratories in the country. The Laboratory of Genetics, Physiology and Reproductions is supervised by Professor Eliana Zelada, who is interested in collaborating on future conservation genetics projects. Furthermore, with this project, I was able to apply for the XI Workshop on Conservation Genetics (link) conducted by the Conservation Genetic Network. This was an excellent opportunity for me as the workshop focused not only on the scientific components (molecular and genetics theory and analysis) but also included topics such as society and society's relationship with conservation.

3. Environmental education:
   We were able to conduct workshops at primary schools mainly in the city of Ilo, Moquegua which is of particular interest given proximity to a protected area. More than 300 children received information about marine biodiversity, threats to the marine environment and how they can participate in marine conservation. As part of the talks, we conducted games related to topics in marine biology. We originally intended to conduct short evaluations with the participants after the talks, but this was not possible, and we decided to spend more time answering their questions. During our visits we were also able to provide teachers with materials to reinforce their lectures about the marine environment. We remain in contact with three schools from Ilo which are interested in developing an environmental education programme. We also remain in contact with the teacher Milagros Villanueva who has expressed her willingness to help us on similar projects in the future.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

The involvement of members of local communities played an important role in the project. Francisco Bernedo, a fisher from Ilo was part of the team and collaborated in the sample collection along the coast. Fishers from other areas also helped us by providing information about areas with frequent otter sightings. In many cases, they also shared information about marine otter interactions with artisanal fisheries. Furthermore, through this project, we were able to collaborate with other researchers that previously worked with marine otters. This has helped to reinforce local efforts toward marine otter conservation.

The collaboration with school headmasters and teachers was also important, especially in the city of Ilo where school authorities were particularly open and helpful during the talks. Some teachers also requested further information about other marine environment and conservation topics to reinforce their lectures and environmental projects.

5. Are there any plans to continue this work?

Yes, we are going to develop a specific agreement with Professor Eliana Zelada from the National University of Santa to conduct further genetic analysis of the marine otter population but this time employing nuclear DNA markers.

Regarding the environmental education activities, we have an agreement with three primary school from the city of Ilo to collaborate on an environmental programme that will help reinforce their existing initiatives.

6. How do you plan to share the results of your work with others?

We shared preliminary results at the Peruvian Congress of Marine Science (CONCIMAR) held in Chiclayo, Lambayeque in November 2016. We also intend to prepare a manuscript summarizing our research findings for publication in a peer-reviewed journal.

7. Timescale: Over what period was The Rufford Foundation grant used? How does this compare to the anticipated or actual length of the project?

The RSG was used along the year of the project. The main expenses were accrued during field excursions, particularly from urban transportation. The actual funds necessary for some budget items was higher than expected as in many cases we had to use private transportation to access remote sampling areas. Nevertheless, we were able to save project funds on other items as only two persons were required for the field surveys. Finally, Monica Santa Marian helped us by reducing laboratory expenses by optimizing procedures.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Field trips fares	680	500	-100	
Urban transport	200	596	+396	We had to use private transport in certain areas, as public transportation was not available due to the remote characteristics of the terrain
Lodging	2030	1400	-630	We had planned for three-person field teams however, only two persons were necessary per zone.
Food	3240	2160	-1080	Only two persons per field excursion
DNA extraction kits	1130	600	-530	Only two kits were required
Laboratory genetic analysis	1629	1512	-117	
DNA sequencing	700	667	-33	

Workshops material	260	260	260	
Reports and results dissemination	80	80	0	
Total	9949	7775		

9. Looking ahead, what do you feel are the important next steps?

The most important next step will be to conduct nuclear DNA analysis from scat samples. This will deepen in the genetic structure of the marine otter Peruvian population and therefore complement the results we obtained through mitochondrial DNA analysis.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

Yes, we designed and printed a marine otter sticker. We considered this the best option as it lasts longer and generated less paper waste than a leaflet or paper cut-outs. We made this decision in part in response to the large amounts of solid waste we observed along the coast during the project. Stickers are made of good quality material and were distributed to industrial and artisanal fishers, school children and biology and veterinary students. Every project-related presentation displayed the Rufford logo, including at the Conservation Genetic Workshop held in Misiones, Argentina.

11. Any other comments?

The development of this project has helped to gather researchers interested in marine otter conservation and has also helped kick-start other new, small projects focused not only on genetics (i.e. parasites) that will provide additional information about health status of the species.

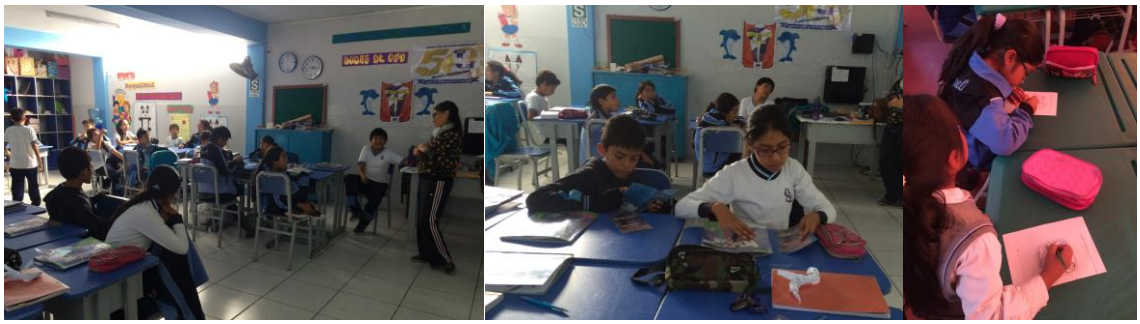

Figure 1: A talk at the Primary school "San Luis" in July 2016.

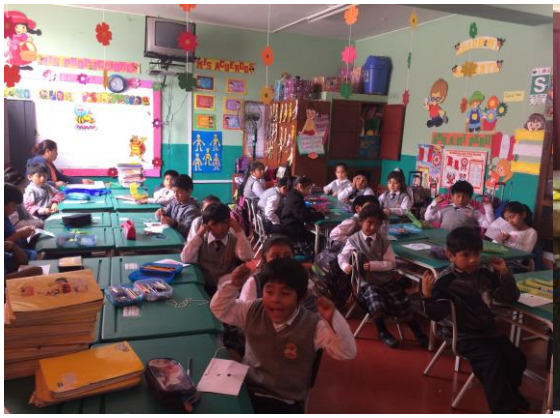
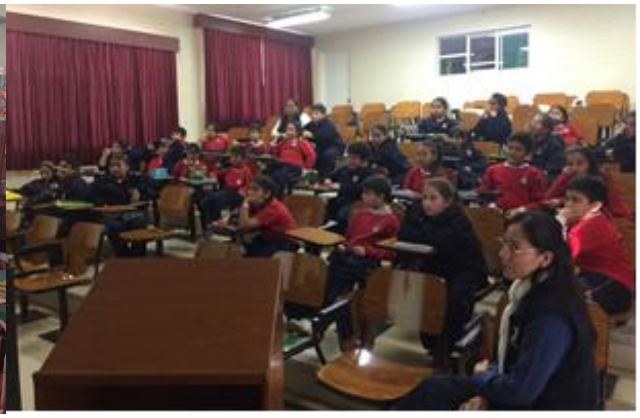

Figure 2: A talk at the National Primary School "Daniel Becerra Ocampo" in October 2016. **Figure 3**: A talk at the Primary School "Enrique Meiggs" in July, 2016.

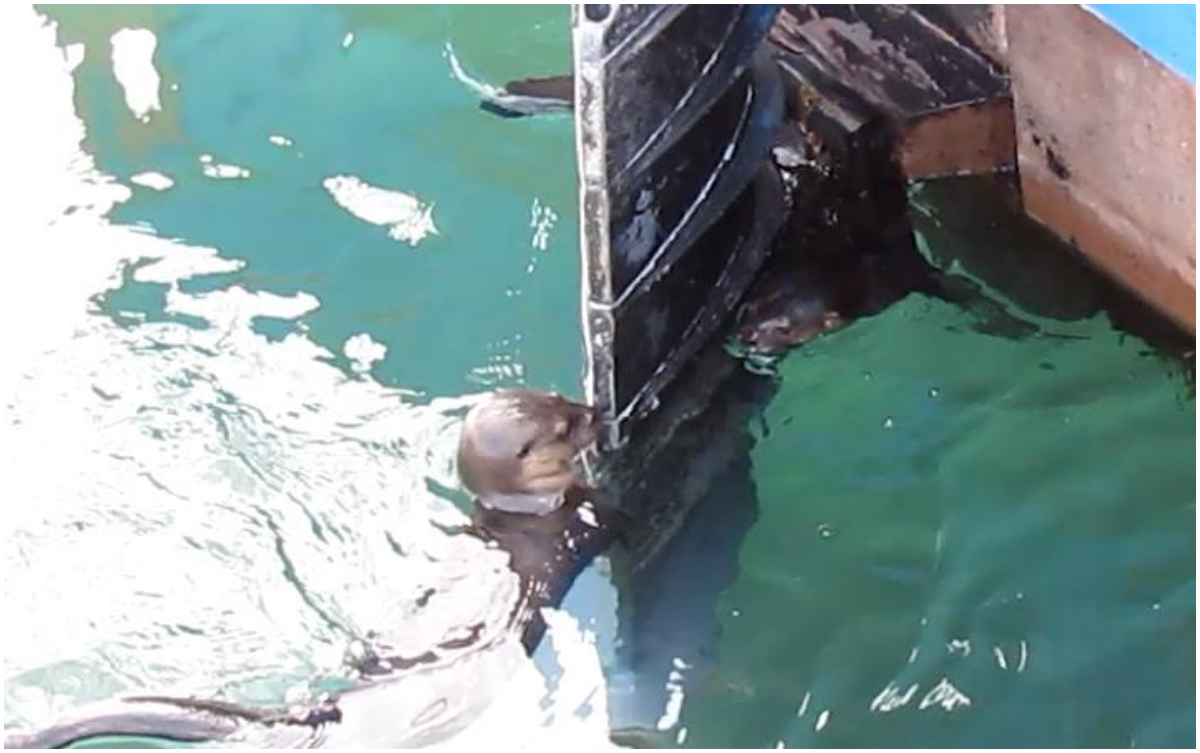

Figure 4: Marine otter spotted close to an artisanal fishing boat in Morro Sama, Tacna.

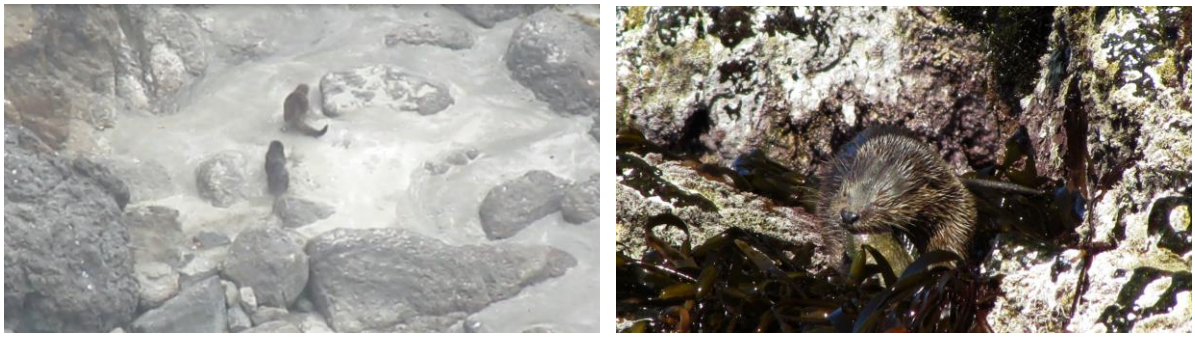

Figure 5: Marine otters spotted in Gramita, Ancash and Tres hermanas, Ica.

Figure 6: Marine otters spotted in San Nicolas, Ica and Catarindo, Arequipa.

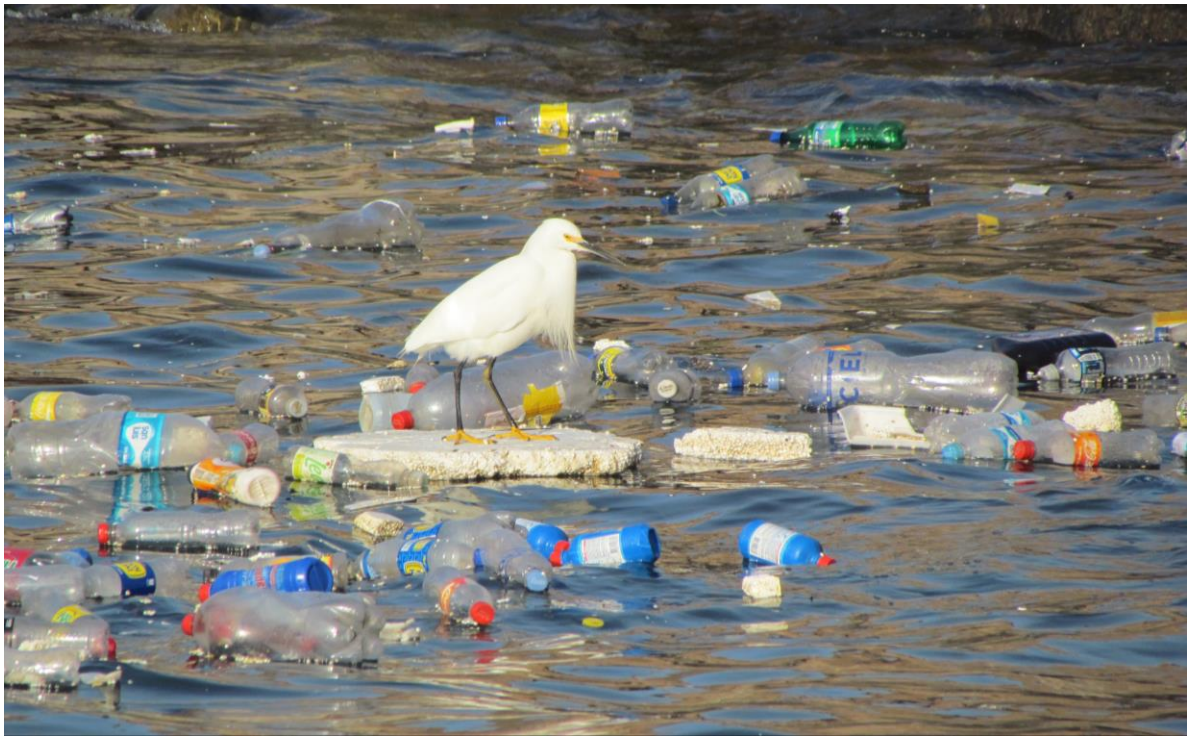

Figure 7: Plastic pollution observed in Matarani, Arequipa.

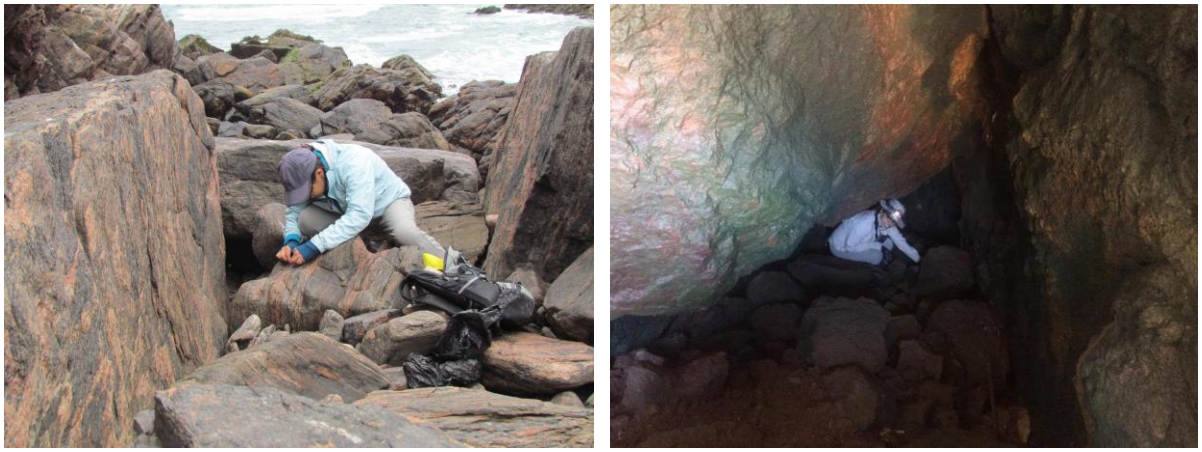

Figure 8: Sample collection in Quilca, Arequipa and San Nicolas, Ica.

Figure 9: A marine otter spotted in Ancon, Lima.

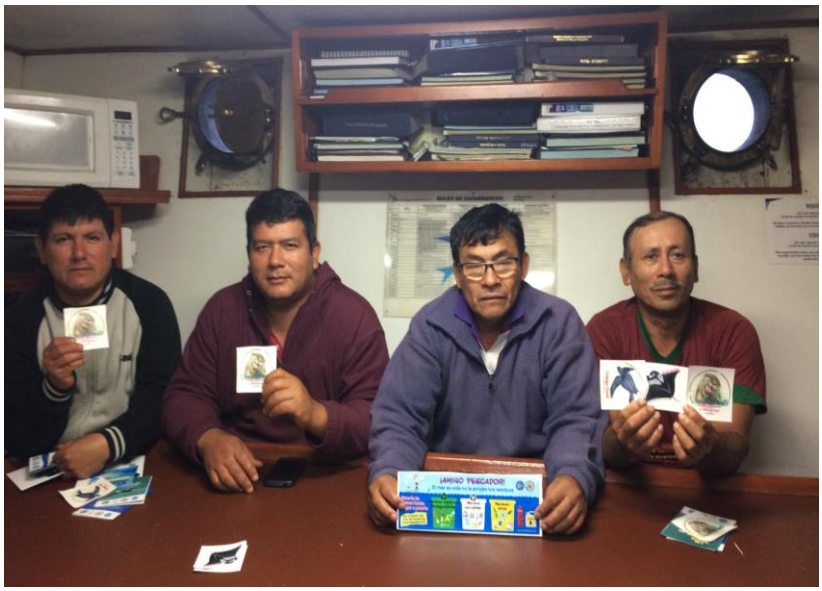

Figure 10: Industrial fishers with marine otter stickers.

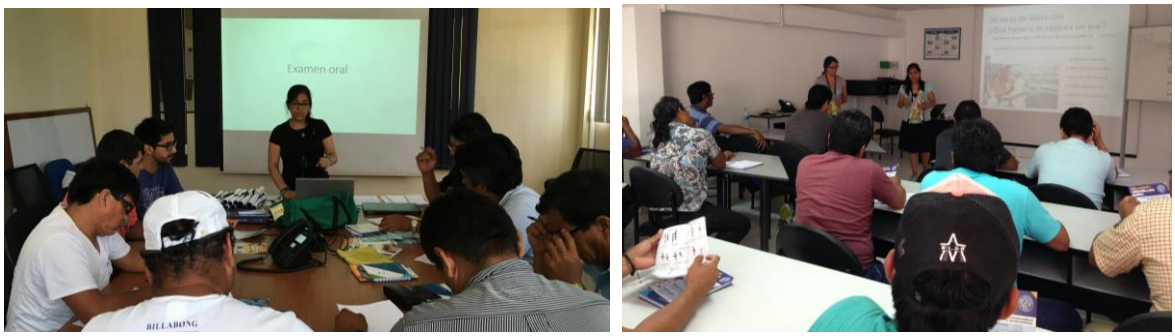

Figure 11: A talk conducted with industrial fishers in February 2017.

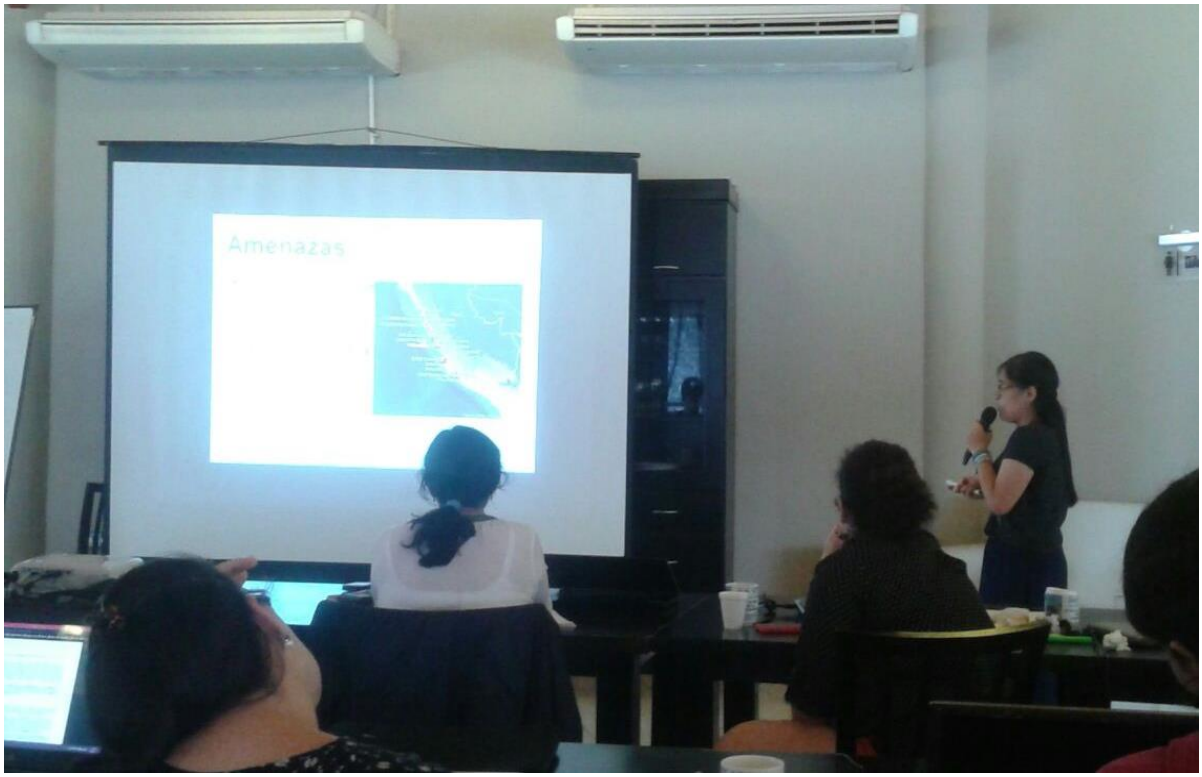

Figure 12: Presenting the marine otter project at the Conservation Genetics workshop held in Misiones, Argentina, February 2017.

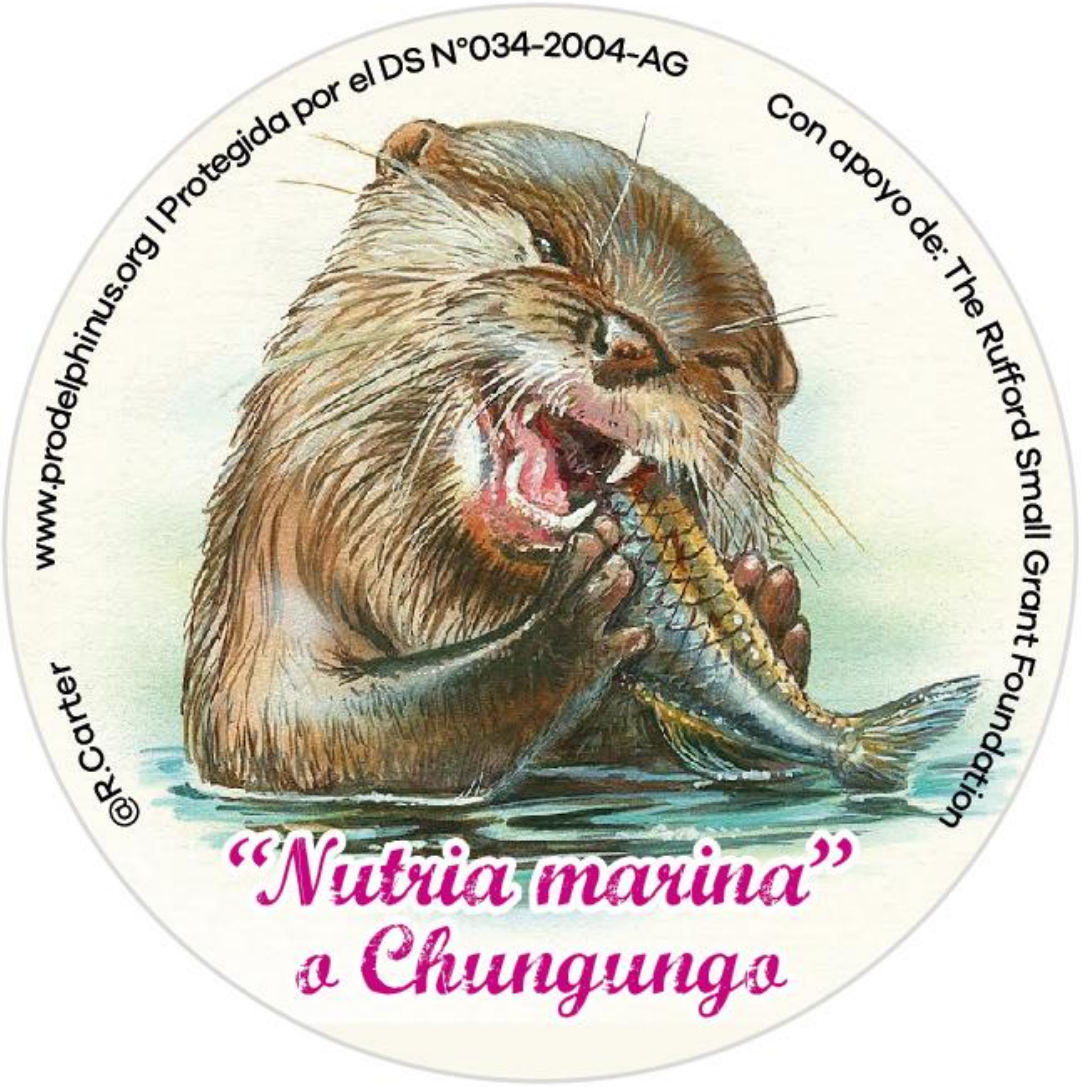

Figure 13: Marine otter sticker designed as part of the project.